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(12) **United States Patent**  
**Murphy et al.**(10) **Patent No.:** **US 8,502,018 B2**  
(45) **Date of Patent:** **\*Aug. 6, 2013**(54) **METHODS OF MODIFYING EUKARYOTIC CELLS**

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(51) **Int. Cl.****A01K 15/00** (2006.01)**A01K 67/027** (2006.01)**C12N 15/63** (2006.01)**C12N 5/00** (2006.01)(52) **U.S. Cl.**USPC ..... **800/18**; 800/8; 800/21; 800/22; 435/325; 435/320.1(58) **Field of Classification Search**USPC ..... 800/18, 8, 21, 22; 435/325, 320.1  
See application file for complete search history.(56) **References Cited**

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**ABSTRACT**

A method for engineering and utilizing large DNA vectors to target, via homologous recombination, and modify, in any desirable fashion, endogenous genes and chromosomal loci in eukaryotic cells. These large DNA targeting vectors for eukaryotic cells, termed LTVECs, are derived from fragments of cloned genomic DNA larger than those typically used by other approaches intended to perform homologous targeting in eukaryotic cells. Also provided is a rapid and convenient method of detecting eukaryotic cells in which the LTVEC has correctly targeted and modified the desired endogenous gene(s) or chromosomal locus (loci) as well as the use of these cells to generate organisms bearing the genetic modification.

**20 Claims, 7 Drawing Sheets**